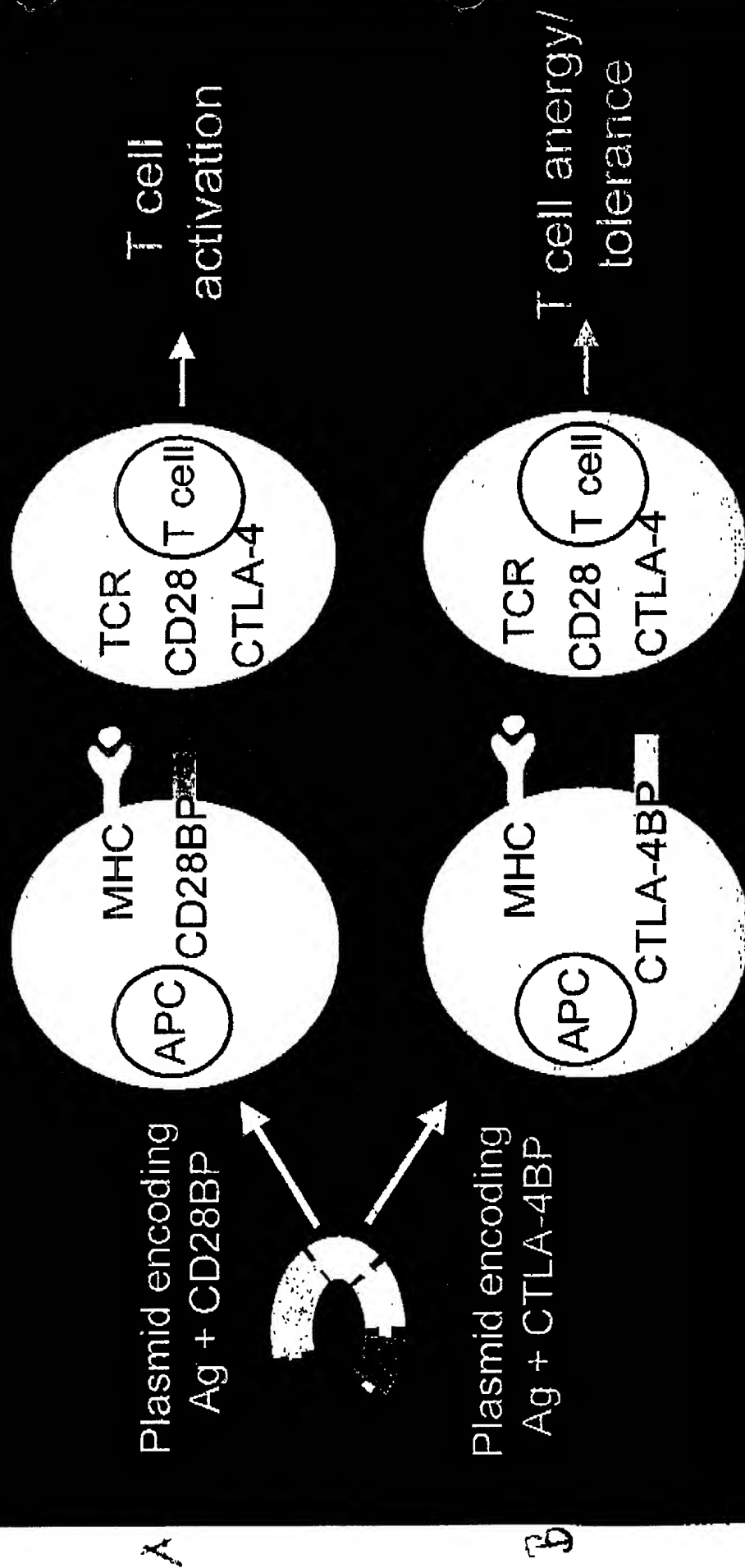


Altered T cell Function Evolved Ligand Binding



MA YGEN

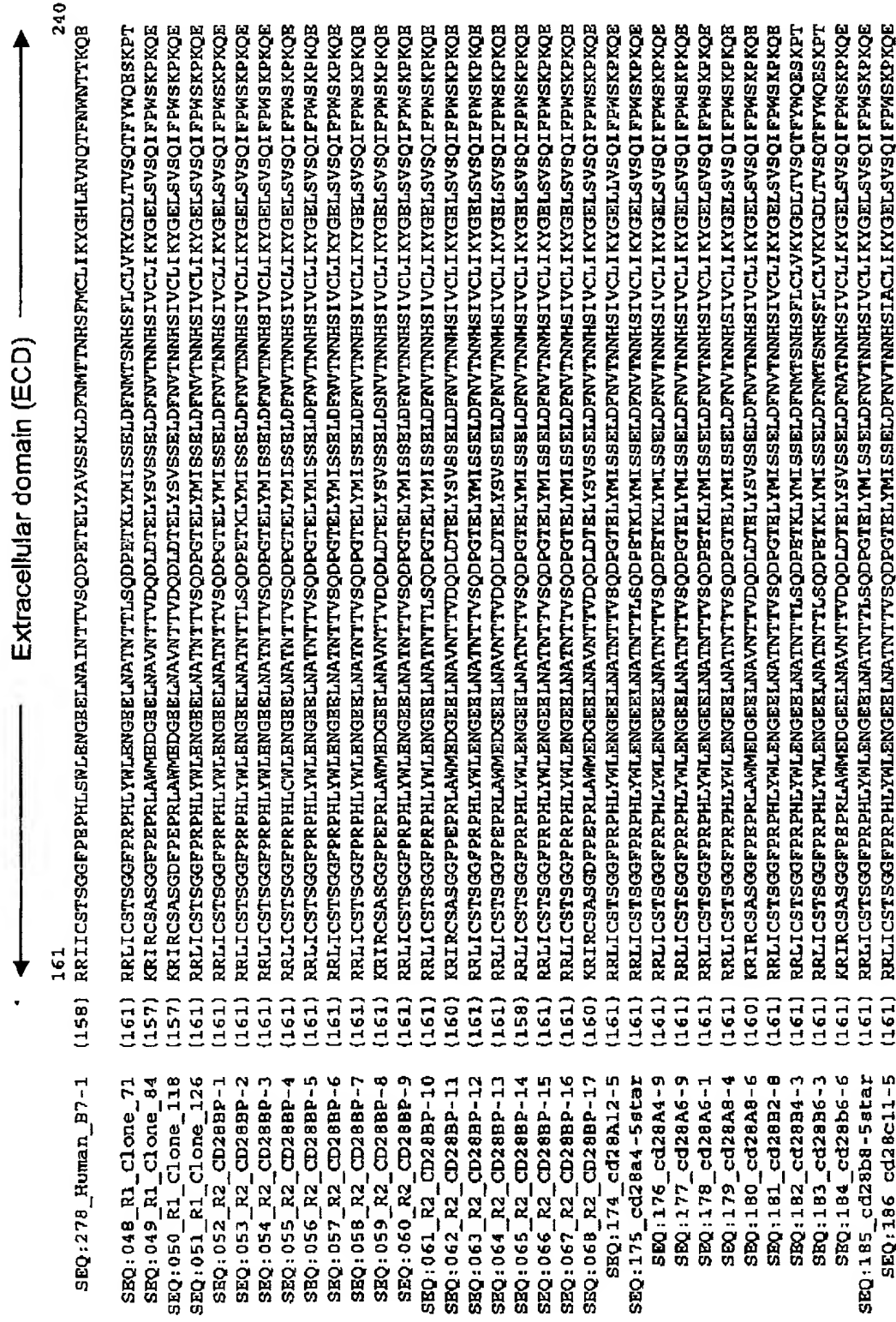


Fig. 2E

	←	Extracellular domain (ECD)	→
161			240
SEQ: 187_cd28C6-1	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDPGTETELYMISSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 188_cd28C7-3	(161)	KRIKCSASGDFEPRRLAWMEDGEEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 189_cd28C8-6	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDPGTETELYMISSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 190_cd28C9-5star	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDPGTETELYMISSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 191_cd28C2-4	(158)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDPGTETELYMISSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 192_cd28D2-3	(159)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDPGTETELYMISSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 193_cd28D2-9	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDPGTETELYMISSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 194_cd28D8-9	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDPGTETELYMISSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 195_cd28D11-1	(160)	KRIKCSASGDFEPRRLAWMEDGEEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 196_cd28D12-5	(161)	KRIKCSASGDFEPRRLAWMEDGEEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 197_cd28E10-6	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDPGTETELYMISSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 198_cd28F7-2	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDPGTETELYMISSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 199_cd28F8-4	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDPGTETELYMISSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 200_cd28F10-2	(161)	KRIKCSASGDFEPRRLAWMEDGEEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 201_cd28F12-5star	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 202_cd28G2-8	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 203_cd28G1-5	(160)	KRIKCSASGDFEPRRLAWMEDGEEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 204_cd28G1-9	(161)	KRIKCSASGDFEPRRLAWMEDGEEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 205_cd28H4-3	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDPGTETELYMISSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 206_cd28H11-3	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDPGTETELYMISSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 207_cd28H6-6	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDPGTETELYMISSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 208_cd28E2-4	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDPGTETELYMISSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 209_cd28B4-5a	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 210_cd28A2-5a	(161)	KRIKCSASGDFEPRRLAWMEDGEEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 211_cd28B4-5star	(161)	KRIKCSASGDFEPRRLAWMEDGEEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 212_cd28D5-6	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 213_cd28D10-4	(160)	KRIKCSASGDFEPRRLAWMEDGEEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 214_cd28E2-5star	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 215_cd28E5-2	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 216_cd28B8-6	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 217_cd28E9-6	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 218_cd28F3-1	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 219_cd28F3-5	(160)	KRIKCSASGDFEPRRLAWMEDGEEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 220_cd28F3-6	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 221_cd28F11-8	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	
SEQ: 283_CD28BF_Con	(161)	RRLICSTSGGFRPHLYWLENGEELNATNTTVSQDLDTELYSVSSSELDNFNTNNHSIVCLIKYGBLSVSQIFPPWSKPQOE	

Fig. 2F

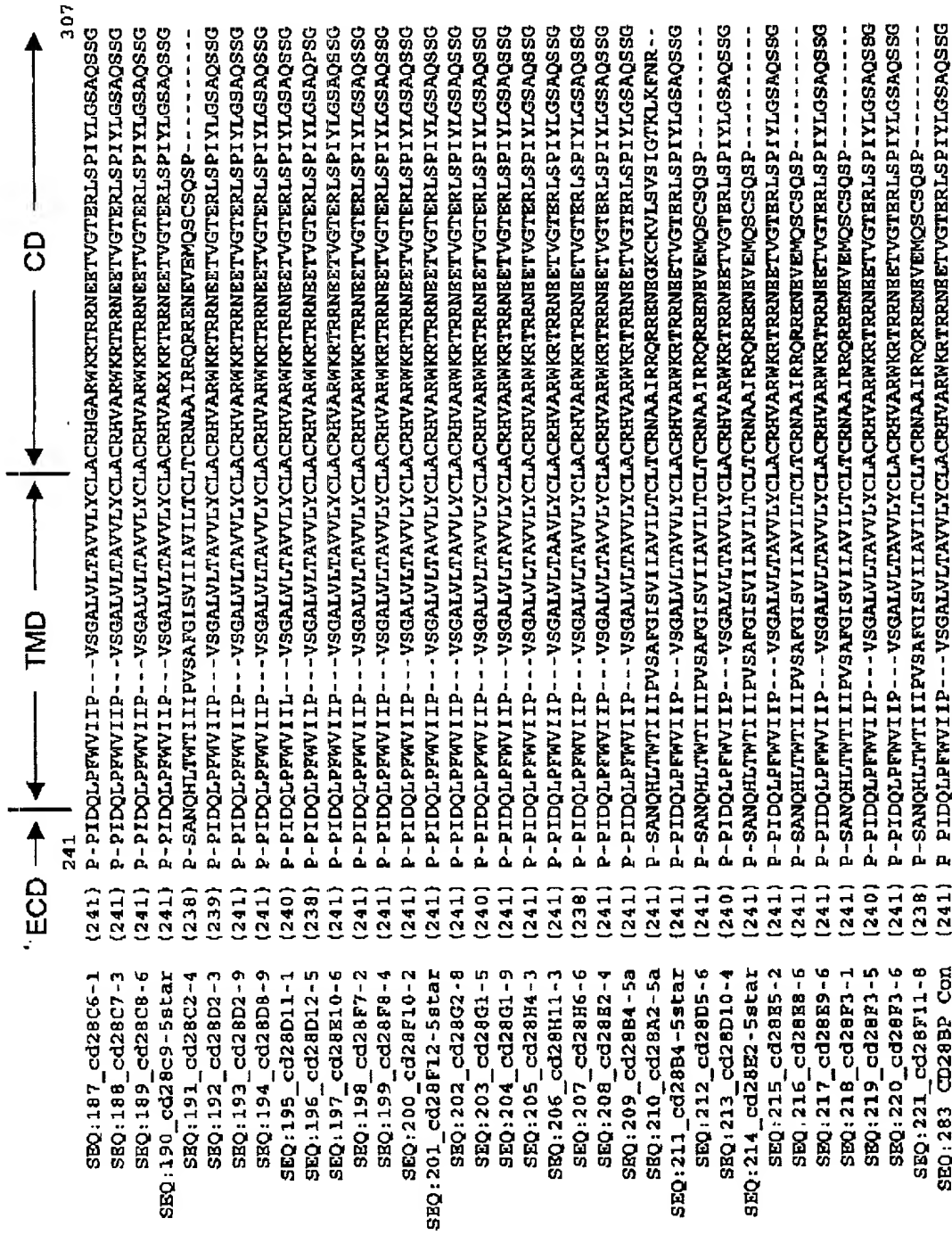


Fig. 2H

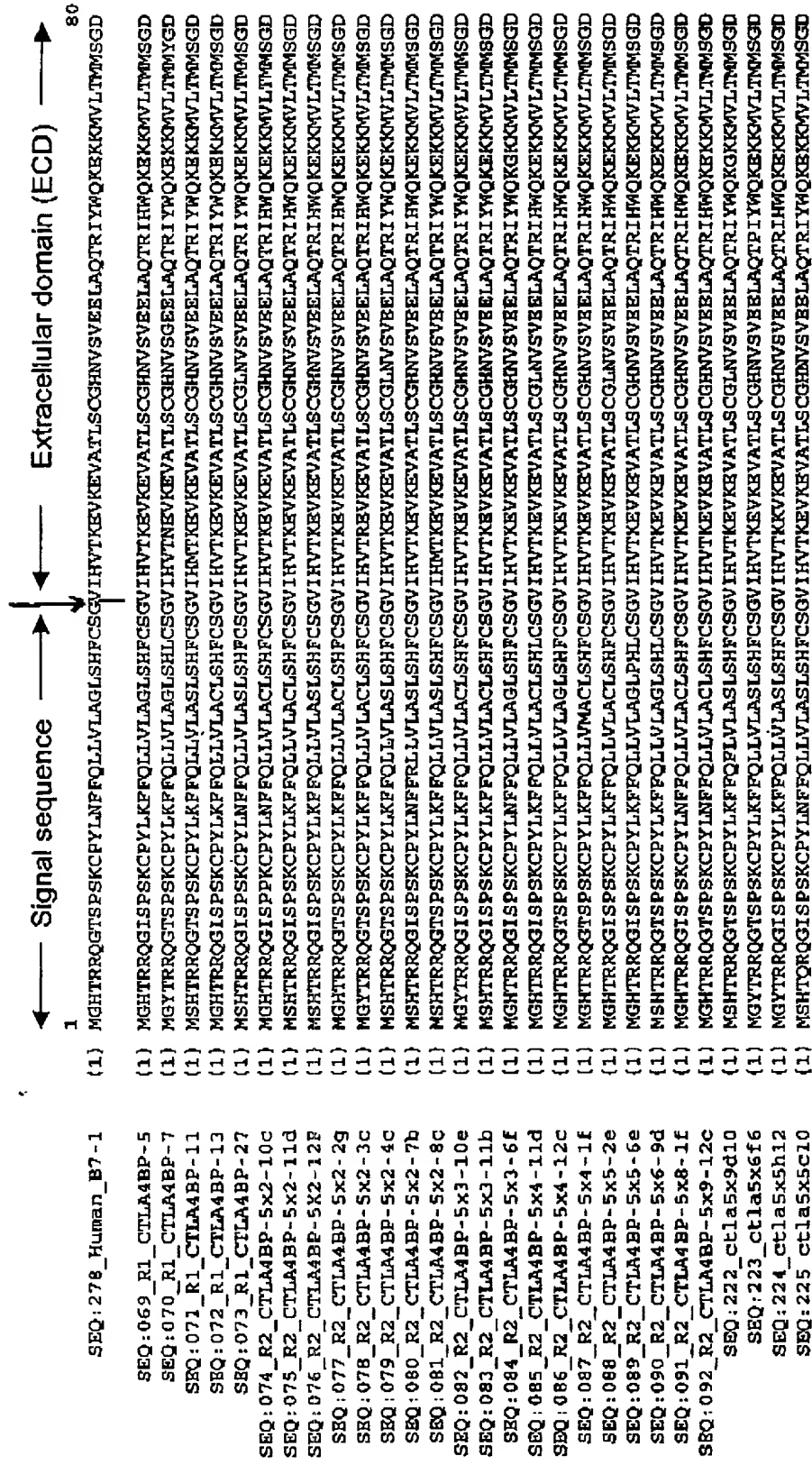
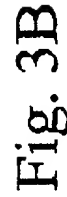


Fig. 3A



[illegible]

Fig. 30

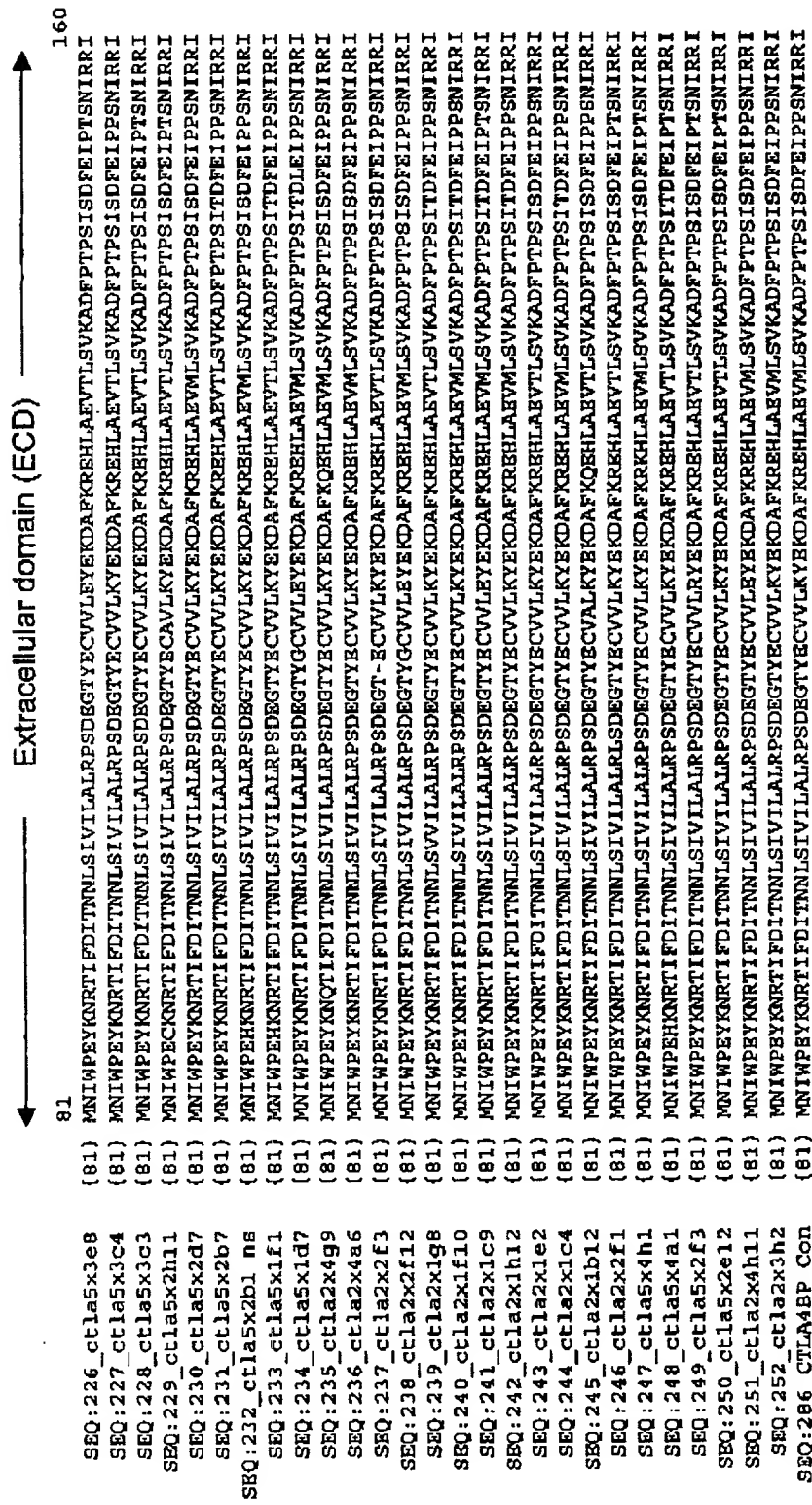


Fig. 3D

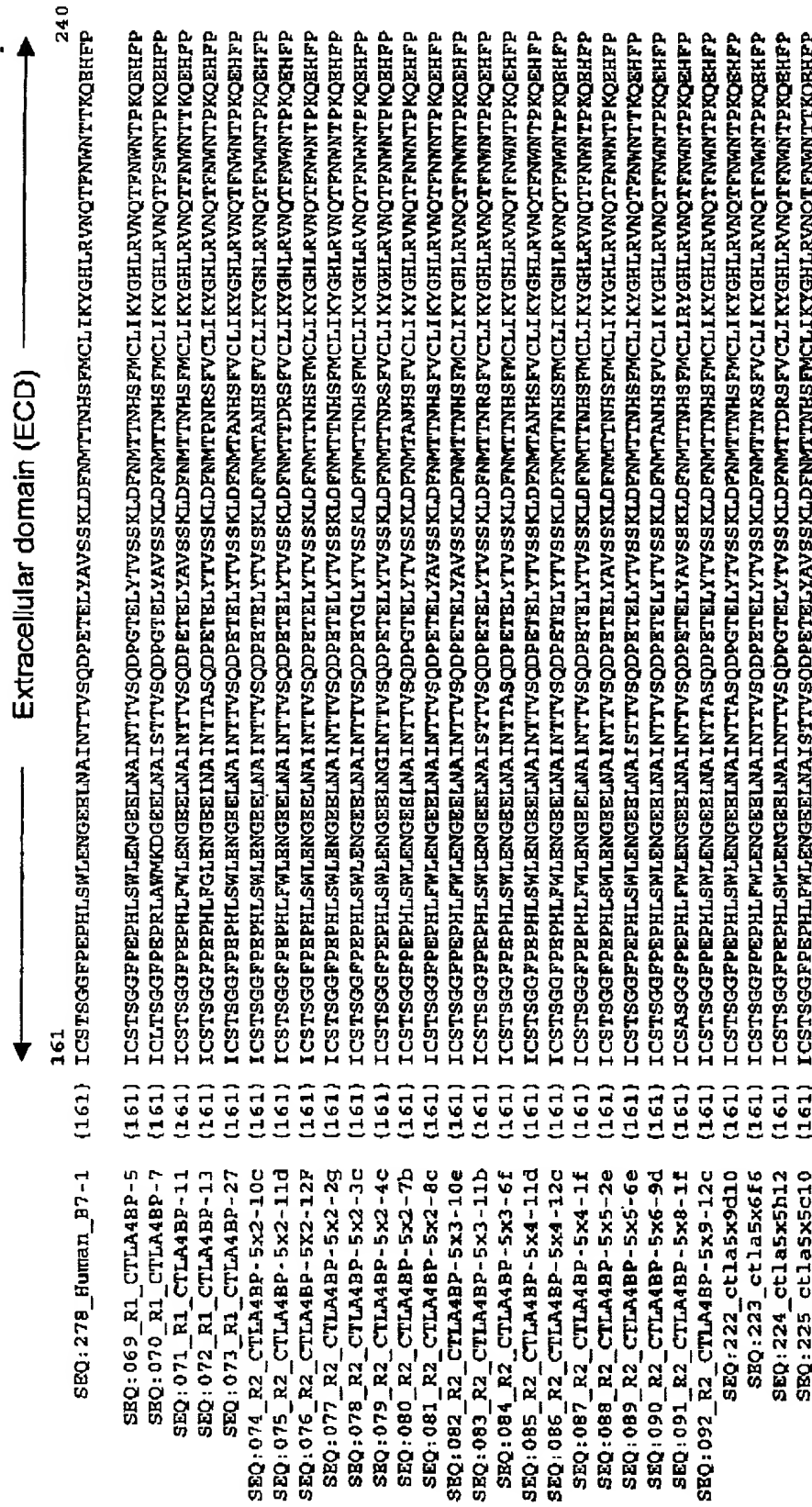


Fig. 3E

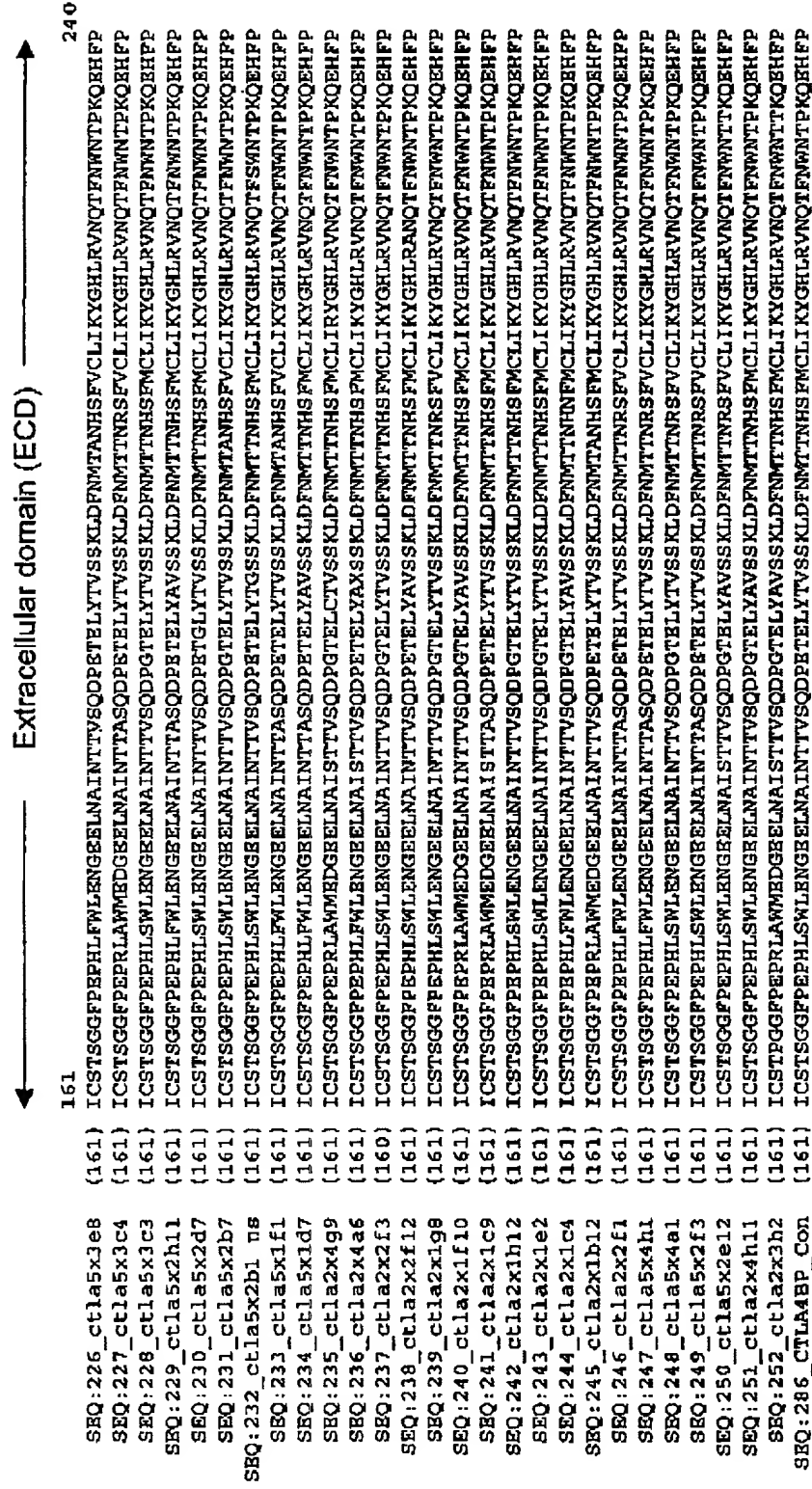


Fig. 3F

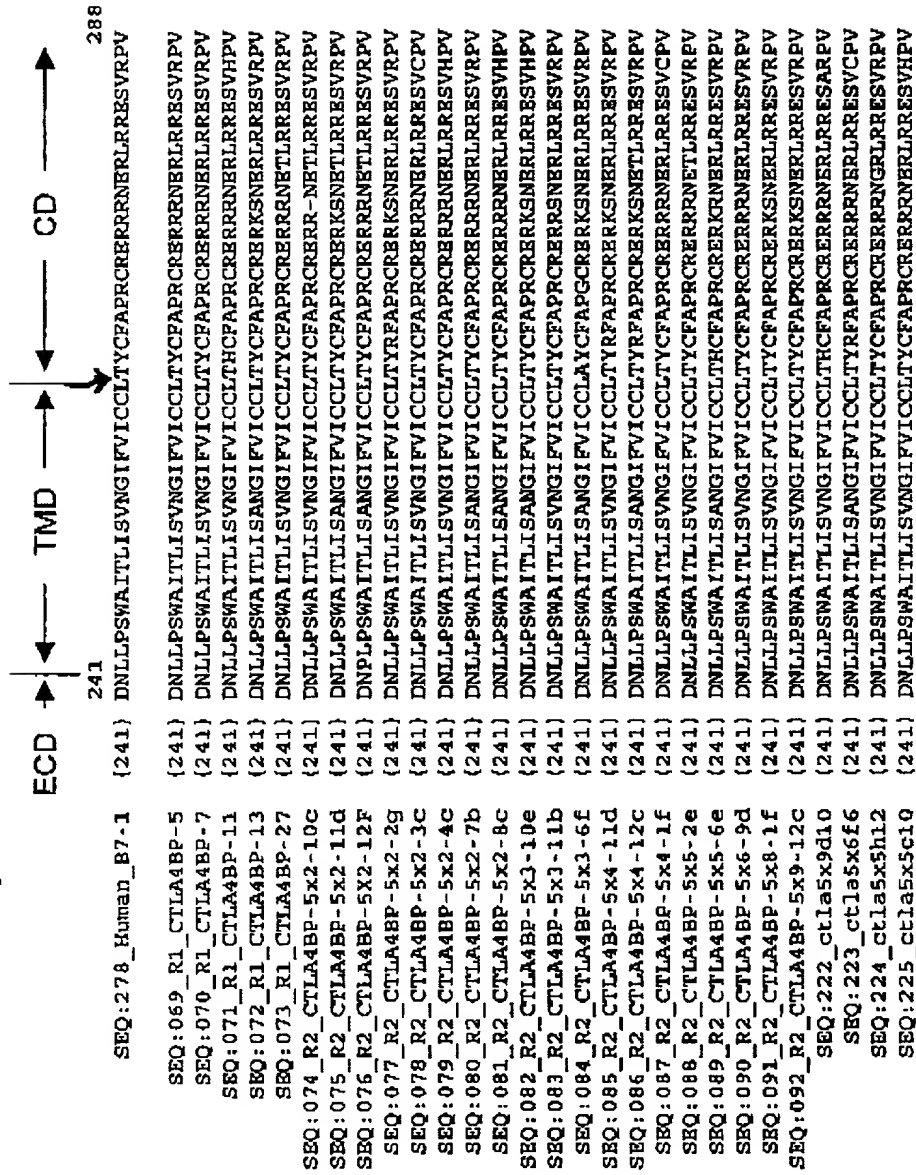


Fig. 3G

Figures 4A-4D

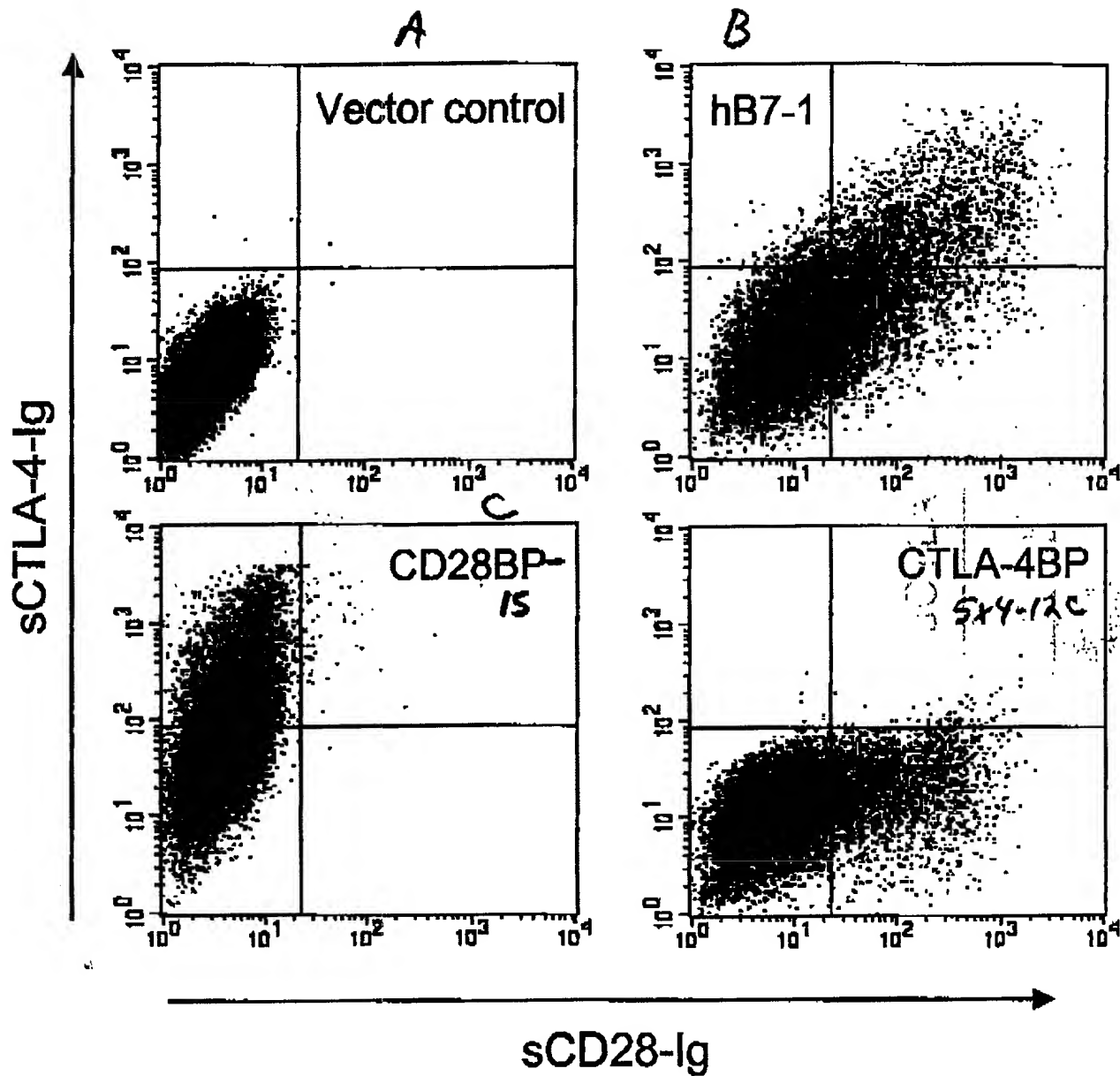
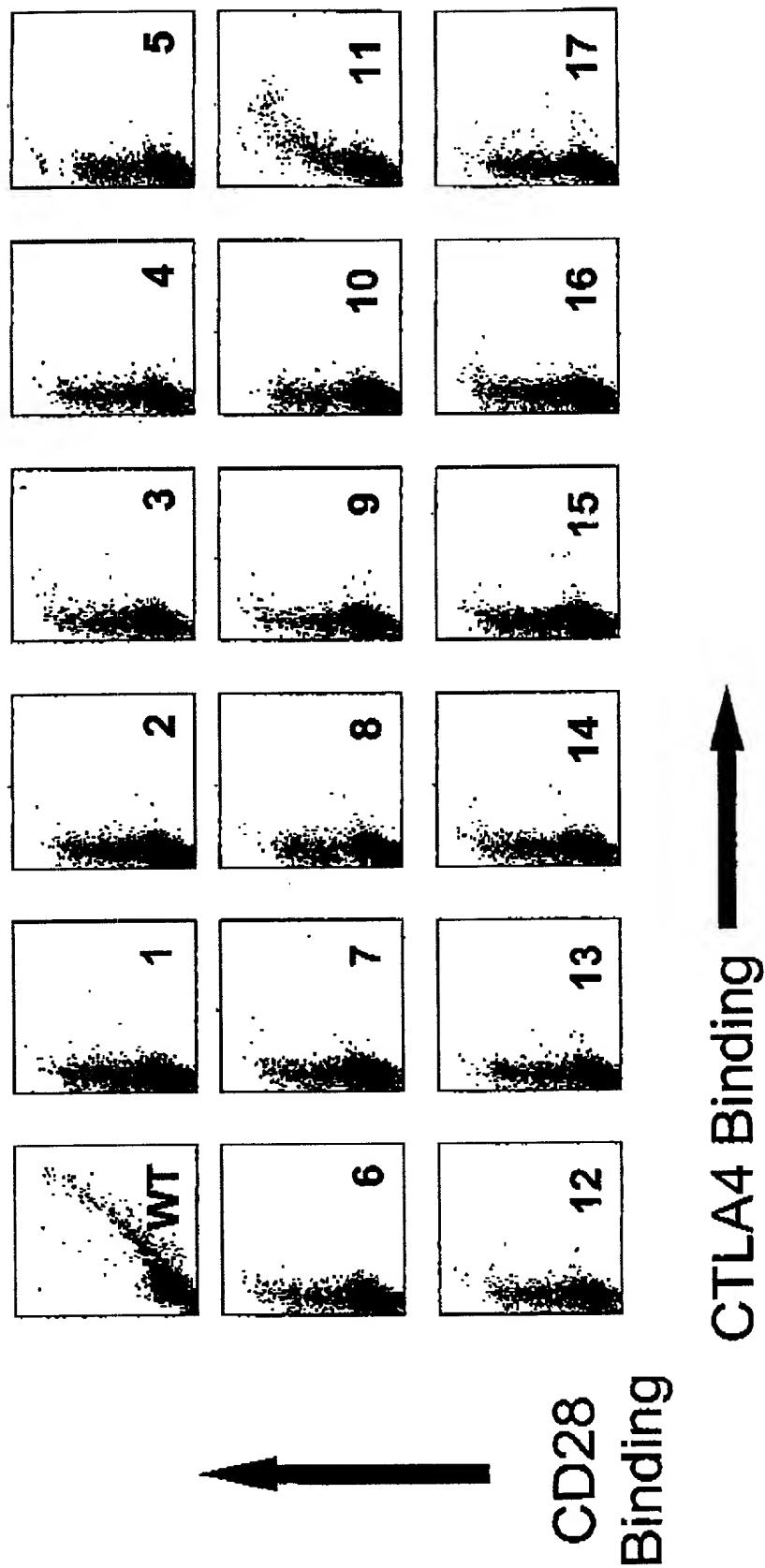


Figure 5

CD28BP after 2nd Round of Shuffling



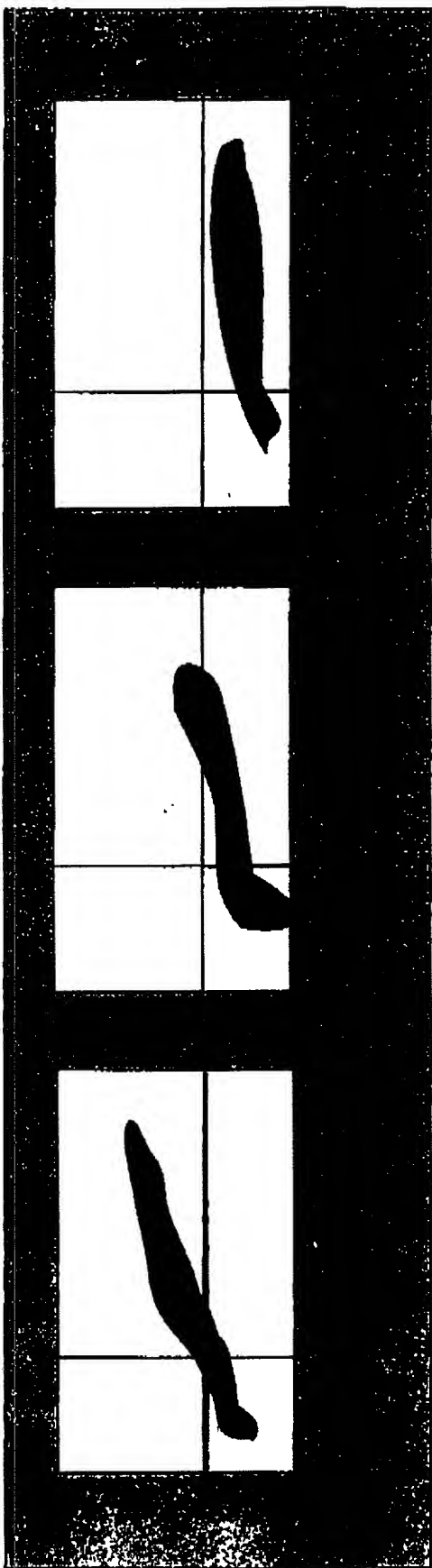
Figures CA-LB.

12345678910

A(1)

A(2)

A(3)

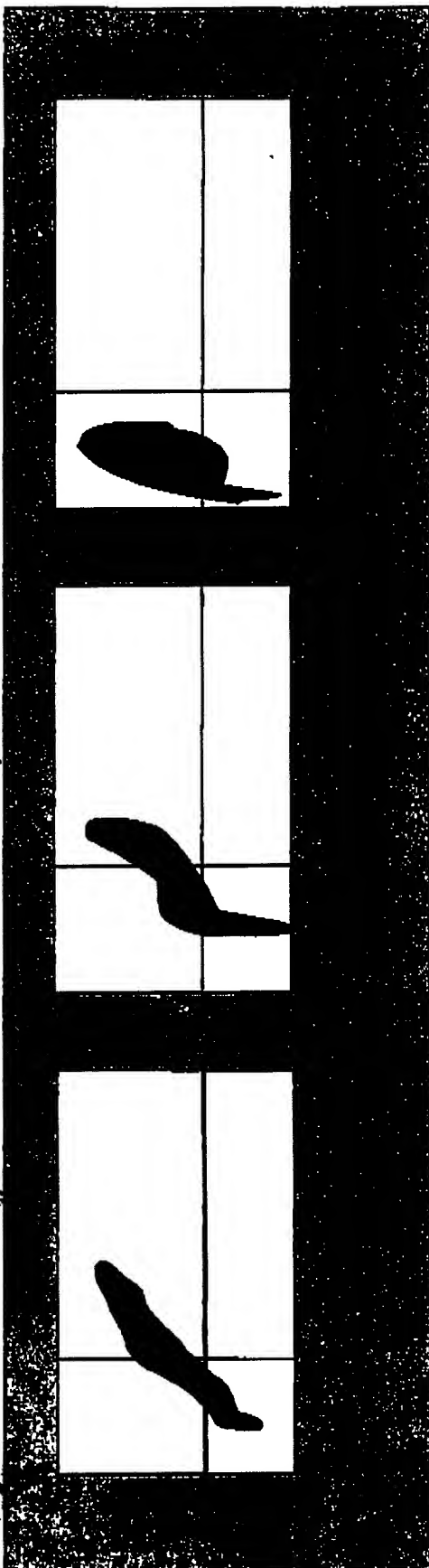


CD28-Ig Binding

B(1) CTLA-4-Ig Binding

B(3)

B(2)

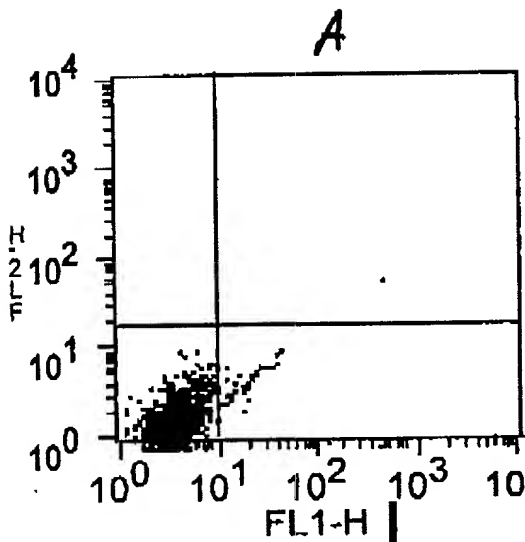


CD28-Ig Binding

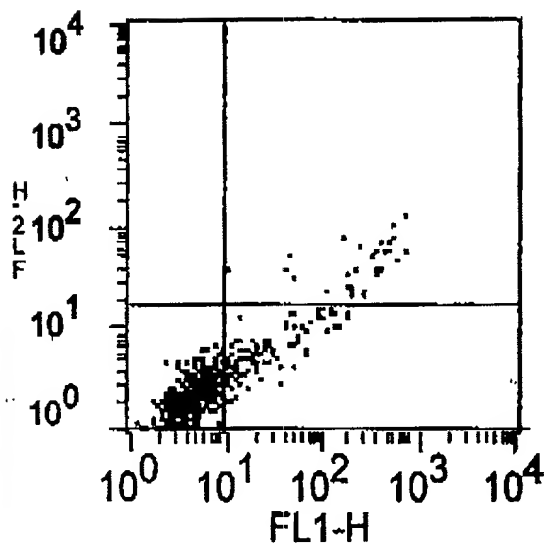
CTLA-4-Ig Binding →

Figures 7A-7D

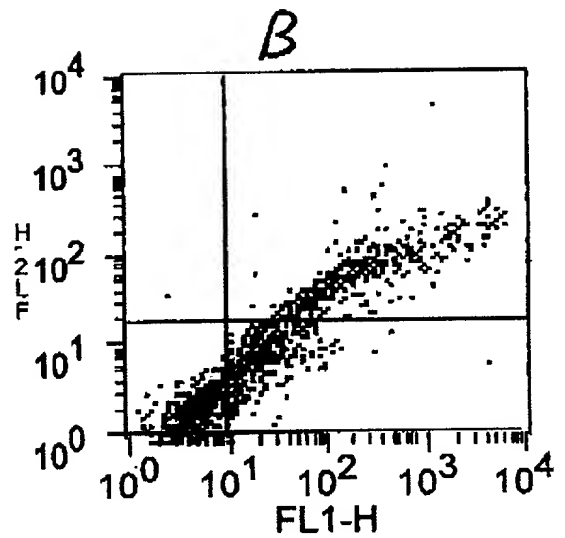
CD28 Binding (PE)



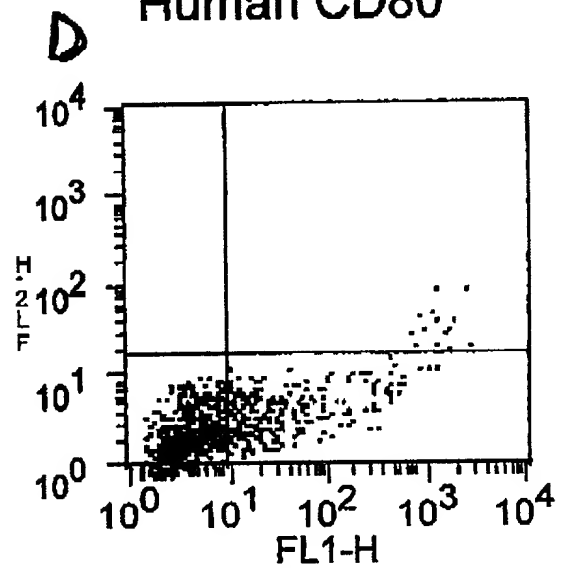
HEK 293



CTLA4BP R25X5-2E



Human CD80

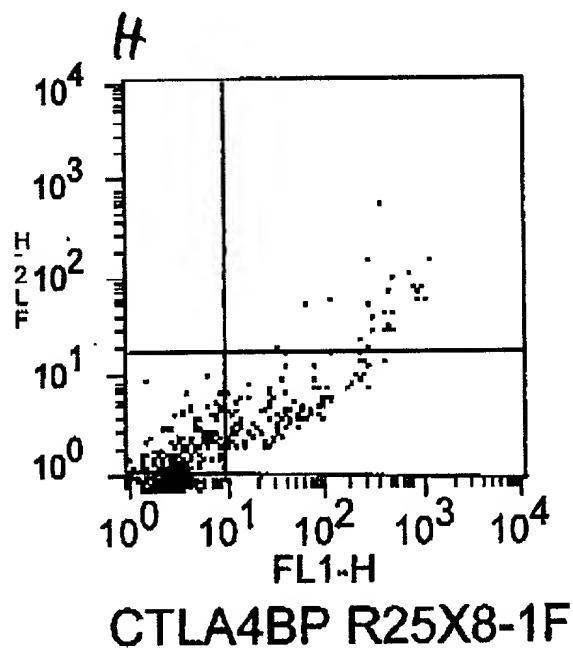
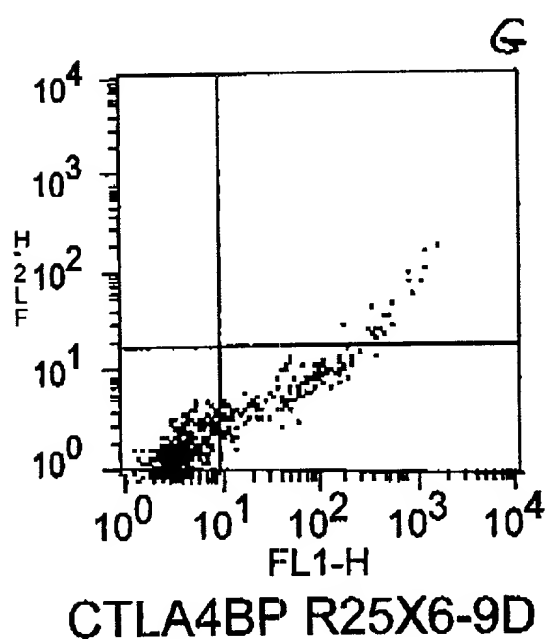
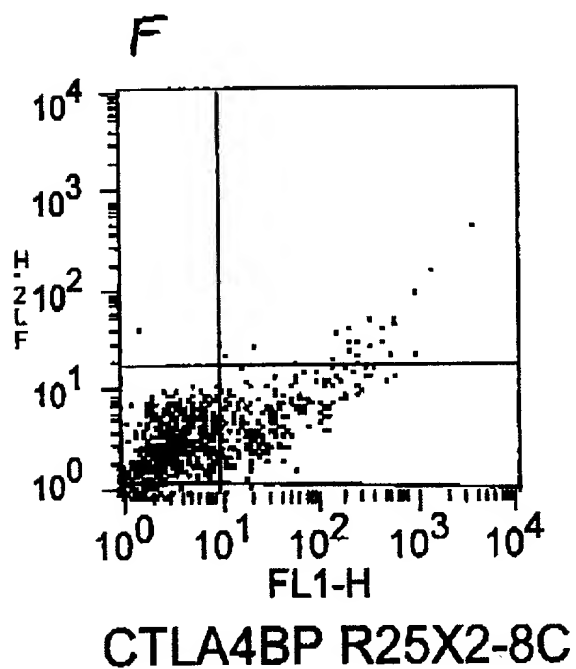
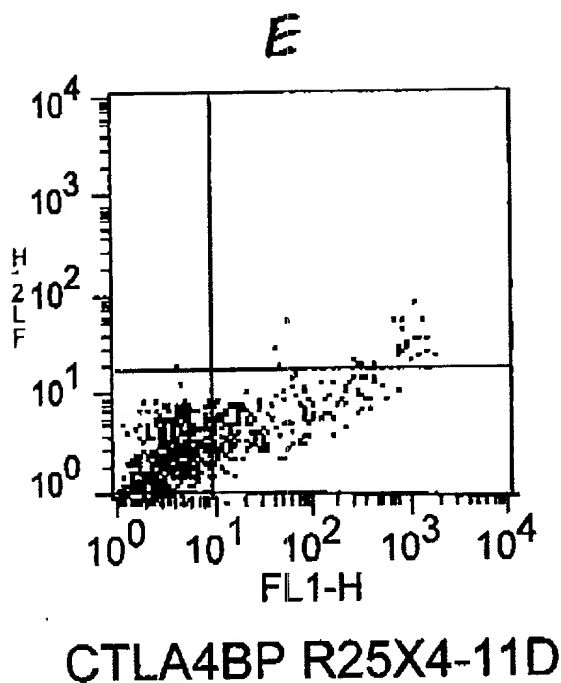


CTLA4BP R25X4-12C

CTLA4 Binding (FITC)

Figures 7E-7H

CD28 Binding (PE)



CTLA4 Binding (FITC)

a CTLA-4BP - 5x4-De

MGHTRRQGTSPSKCPYLKFFQLLVLAGLSHFCSGVIHVTKEVATLSCGHNVSVEELAQT
 K human → baboon → human →
 RIHWQKEKKMVLTMMSGDMNIWPEYKNRTIFDITNNLSIVILALRPSDEGTCECVLKYEKDAF
 human → rhesus/baboon →
 KREHLAEVMSLKADFTPTPSISDFFIPPSPNIRRICSTSGGFPEPHLFWLENGEELNAINTTVSQ
 rhesus/baboon → human → baboon →
 DPETELTVSSKLDENMTTNHSMCLIKYGHIRVNQTFENWNTPKQEHFPDNLPSWAITLISA
 → rhesus
 NGIFVICLTFRFAPRCRERKSNETLRRESVRPV
 human → orangutan → rhesus → baboon

b CD28BP-15

MGHTMKWGSPPKRPCLWLSQLLVLTGLFYFCSGITPKSVTKRVKETVMLS CDYNTSTEELT
 cow →
 SLRIYWQKDSKMVLAILPGKVQVWPEYKNRTITDMNDNPRIVILALRPSDSGTCTYTCVIQKPVLK
 → cow
 GAYKLEHLASVRLMIRADFPVPTINDLGNPSPNIRRLICSTSGGFPRPHLYWLENGEELNAINT
 cow → human → cow
 IVSQDPGTLEYMISSELDENVYNNHSIVCLIKYGELSVSQIFPWSKPKQEPPIQLPFWVPIPV\$
 baboon → rabbit
 GALVLTAVVLYCLACRHVARWKTRRNEETVGTERTLSPIYLGSAQSSG
 human → rhesus/baboon → cow → rabbit →
 orangutan
 rhesus
 baboon

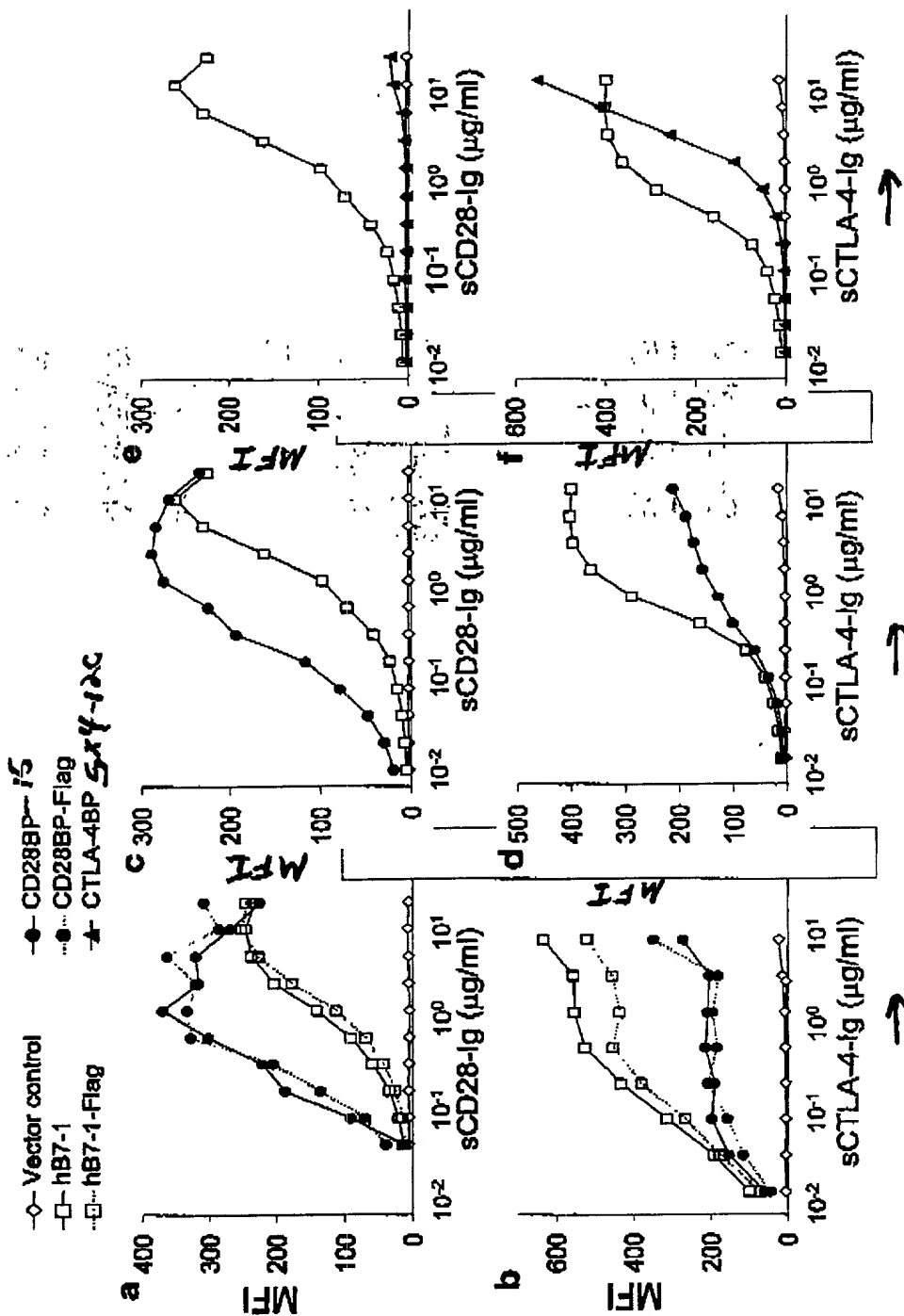


Figure 9A-9H

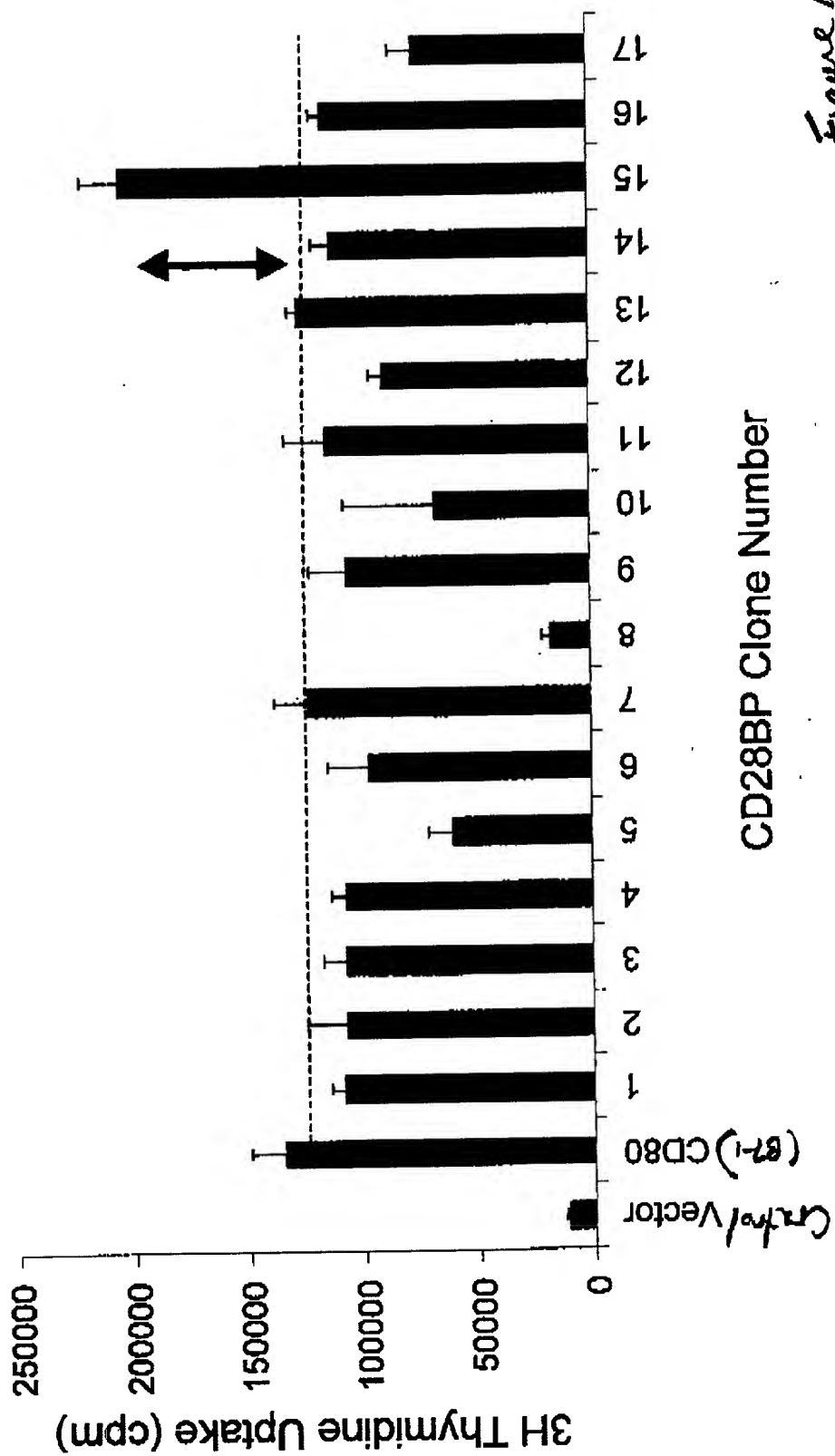
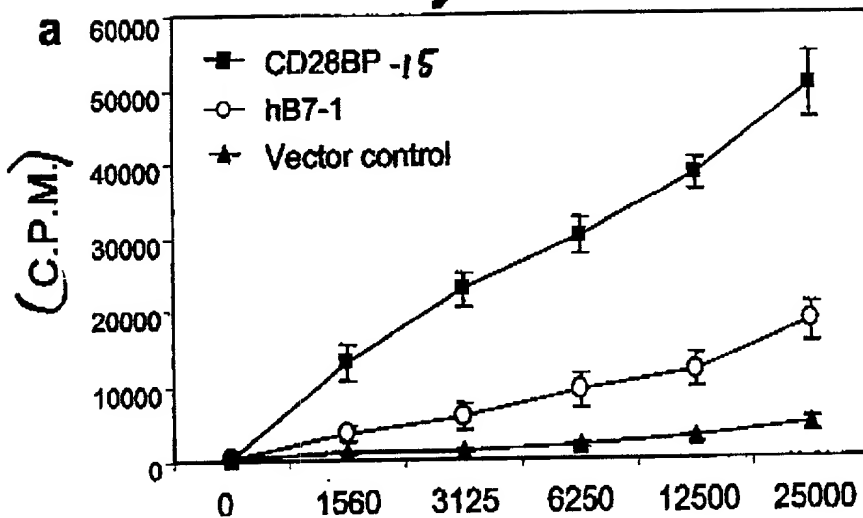


Figure 10

Figures 11A-11C

^3H Thymidine Uptake



^3H Thymidine Uptake

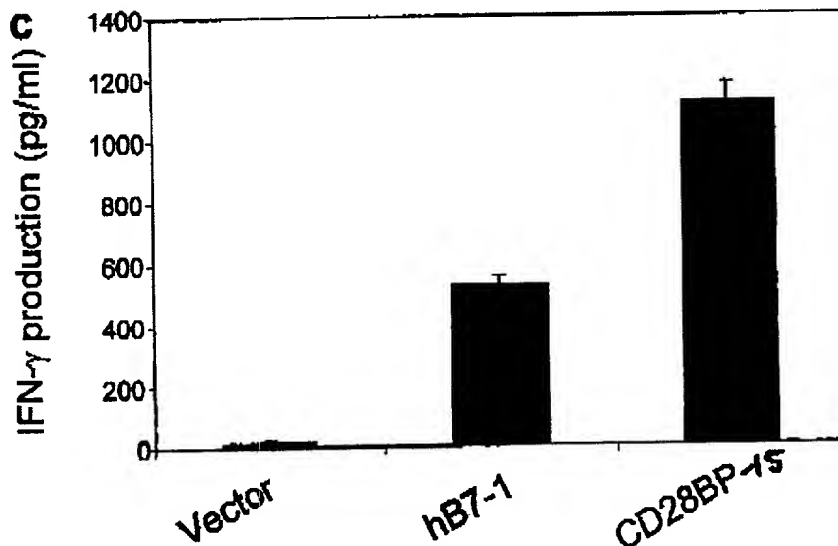
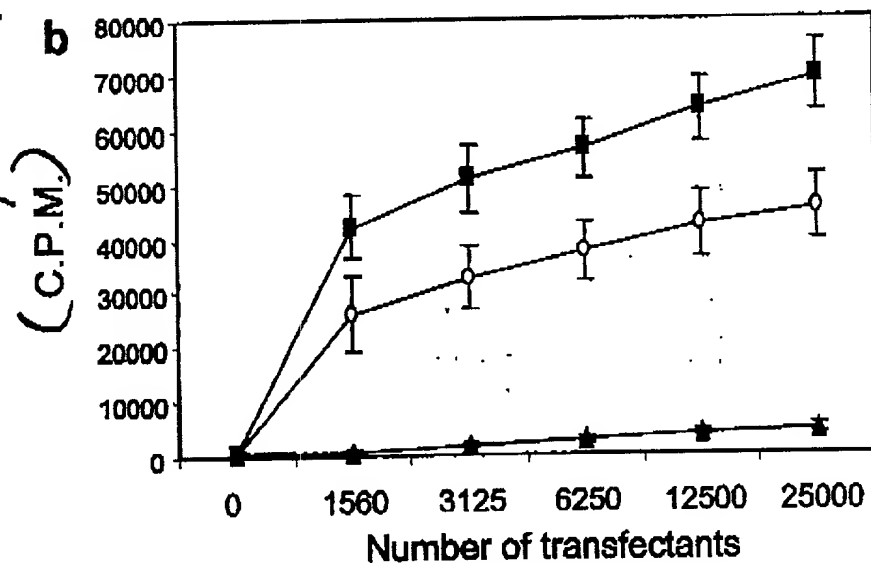
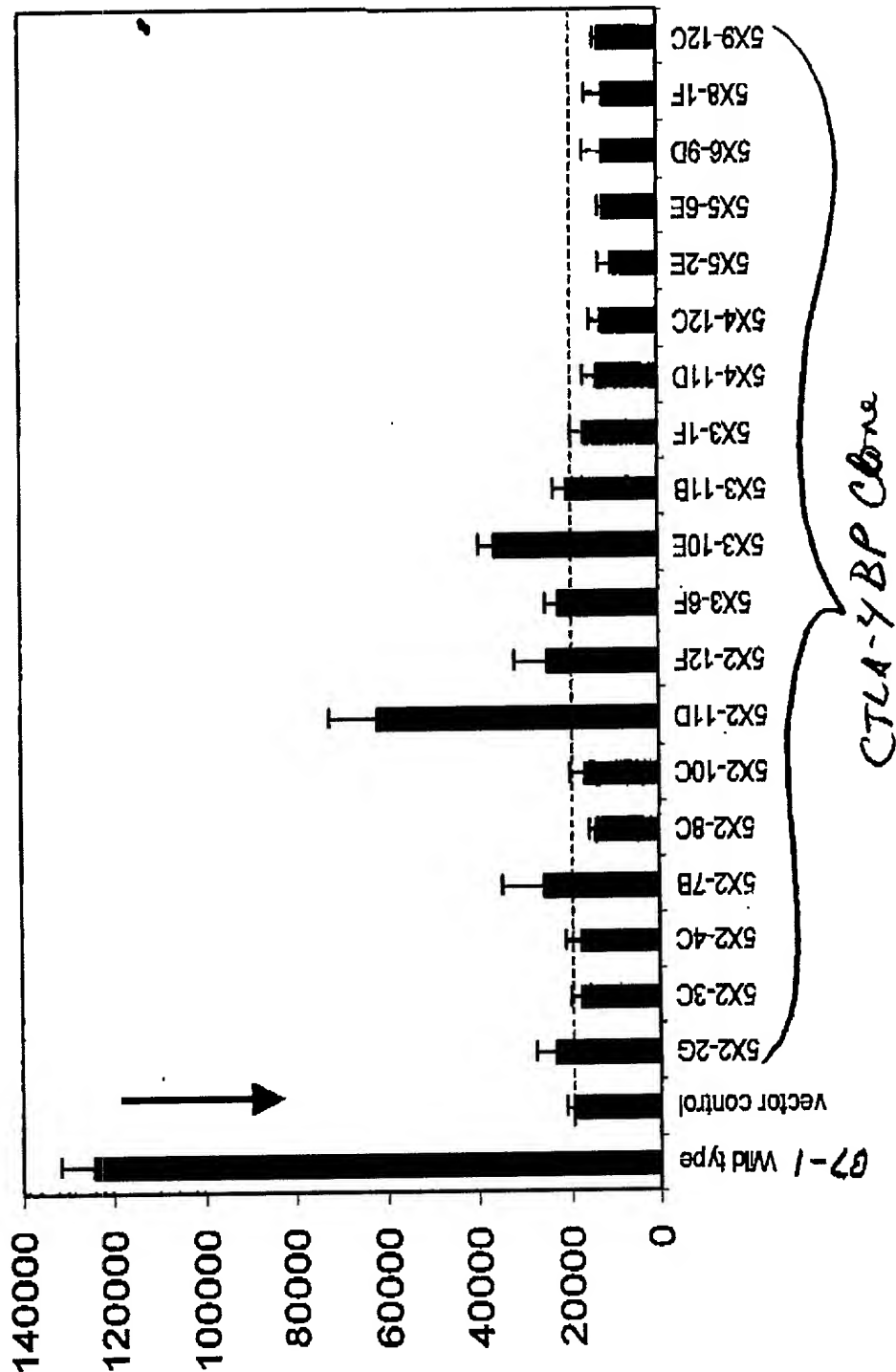


Figure 12

Suppressed T cell Response by CTLA4BP

³H Thymidine Uptake (cpm)

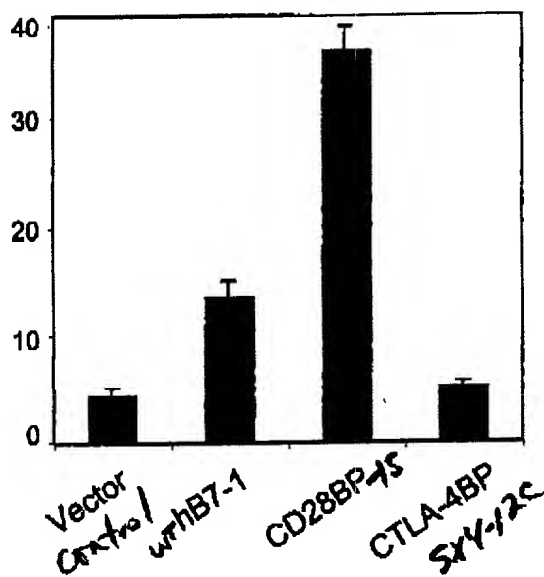


Figures 13A-13D

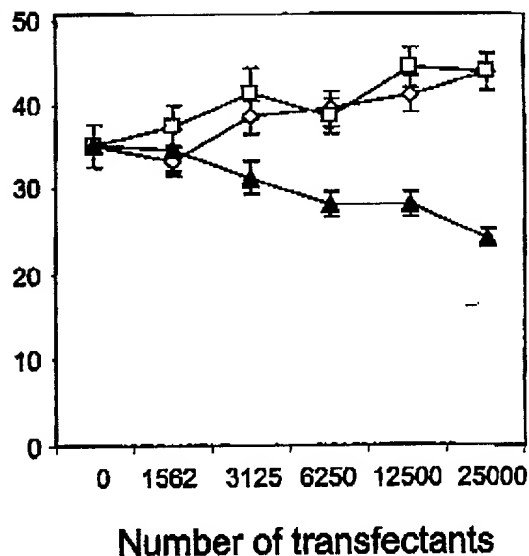
³H Thymidine Uptake

a

C.P.M. (x10⁻³)

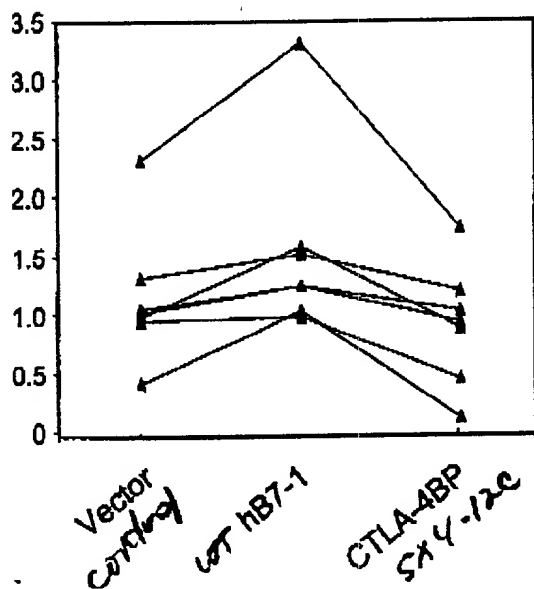


b



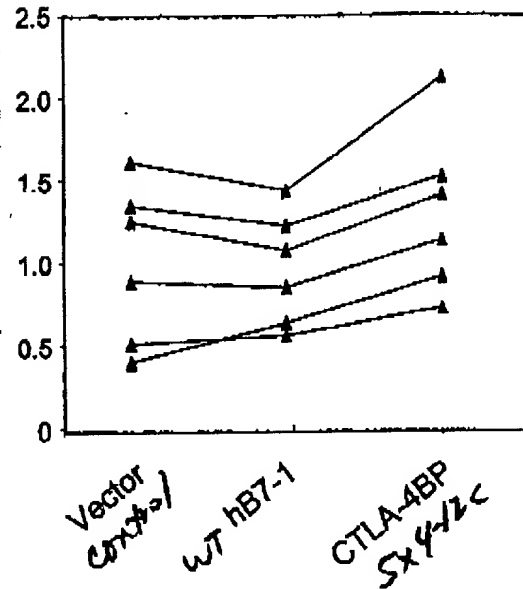
c

IFN- γ production (ng/ml)



d

IL-10 production (ng/ml)



Soluble Forms

A

Human B7.1 sECD

AAAGAPVPYDPLEPR AAHHHHH



Signal	Extracellular Domain	E-epitope His-tag
(1- 34)	(35-242)	(243-259) (260-268)

B Human B7.1 ECD-Ig Form

BstII hinge -CH2-CH3



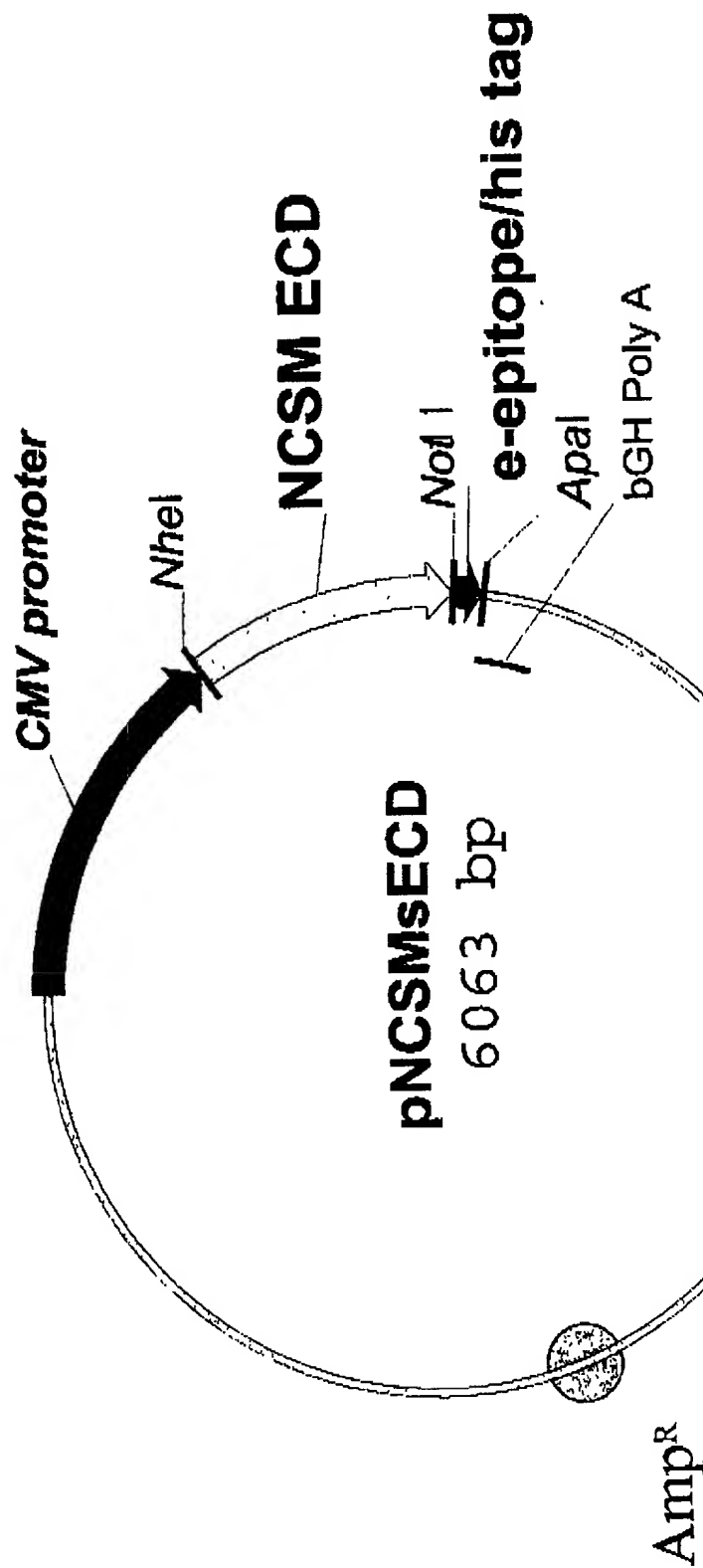
B7.1 ECD (35-242)

Human IgG1 Fc Fragment
GenBank Acc.# P01857
Factor X_a

14A - 14B

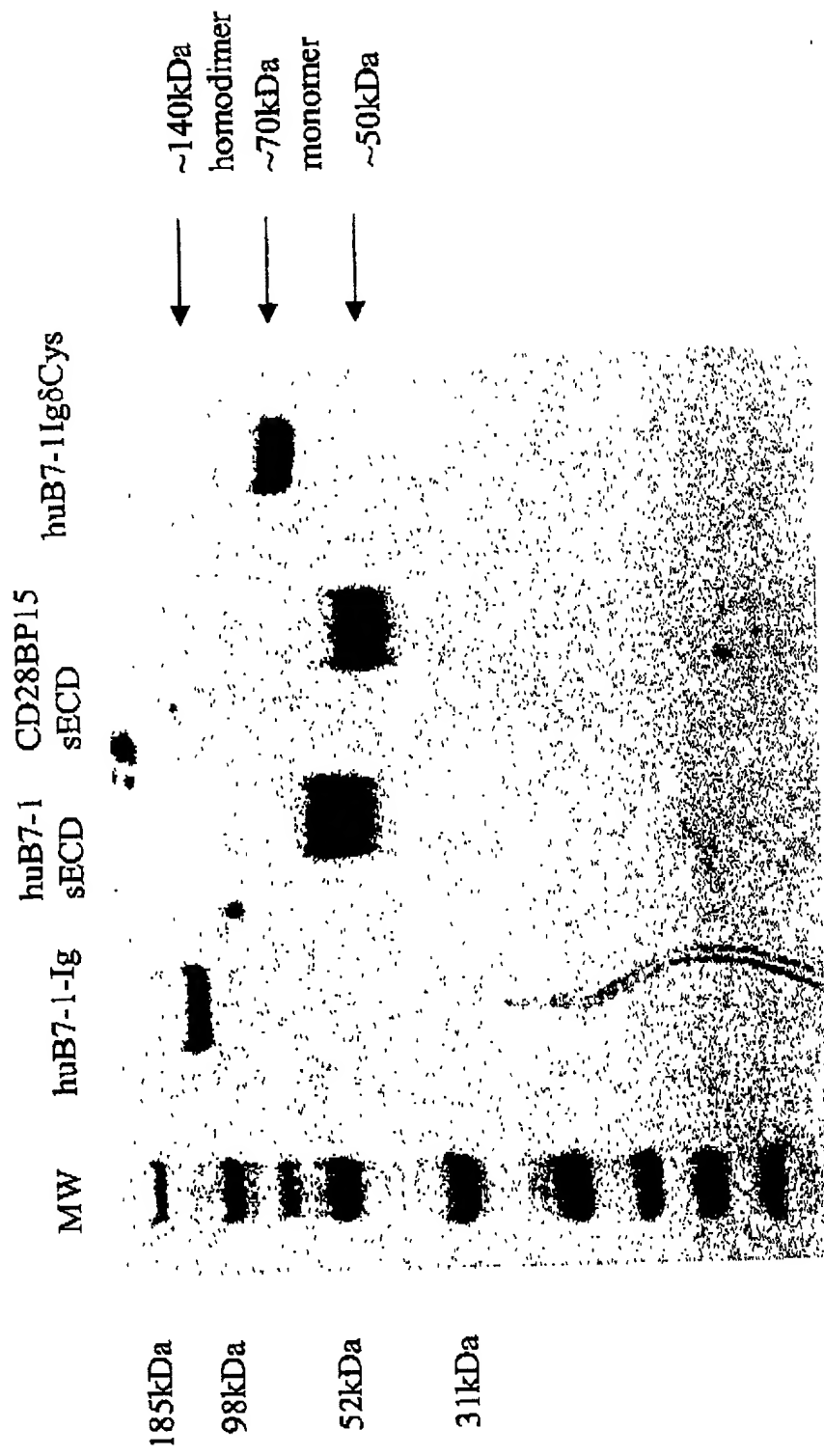
Figures

NCSM-sECD Expression Construct



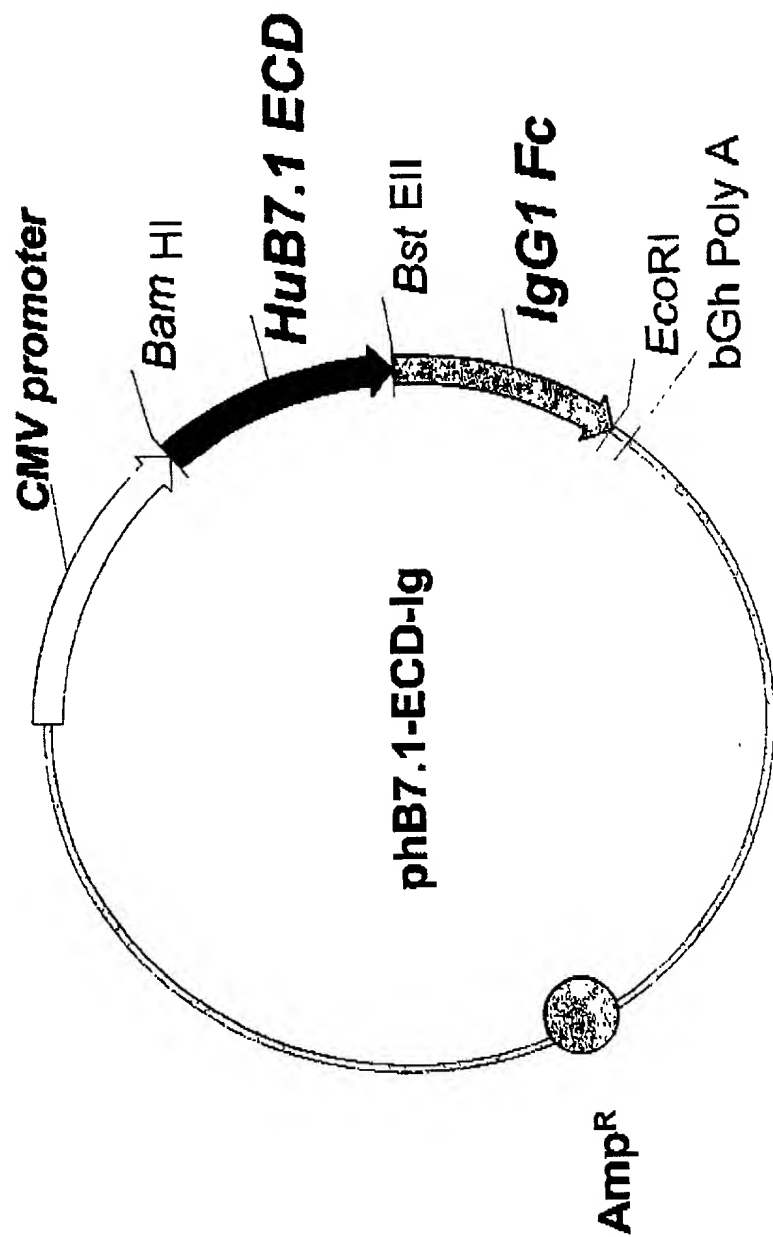
15
Figure 10

SDS-PAGE showing various soluble forms of wt & NCSM proteins

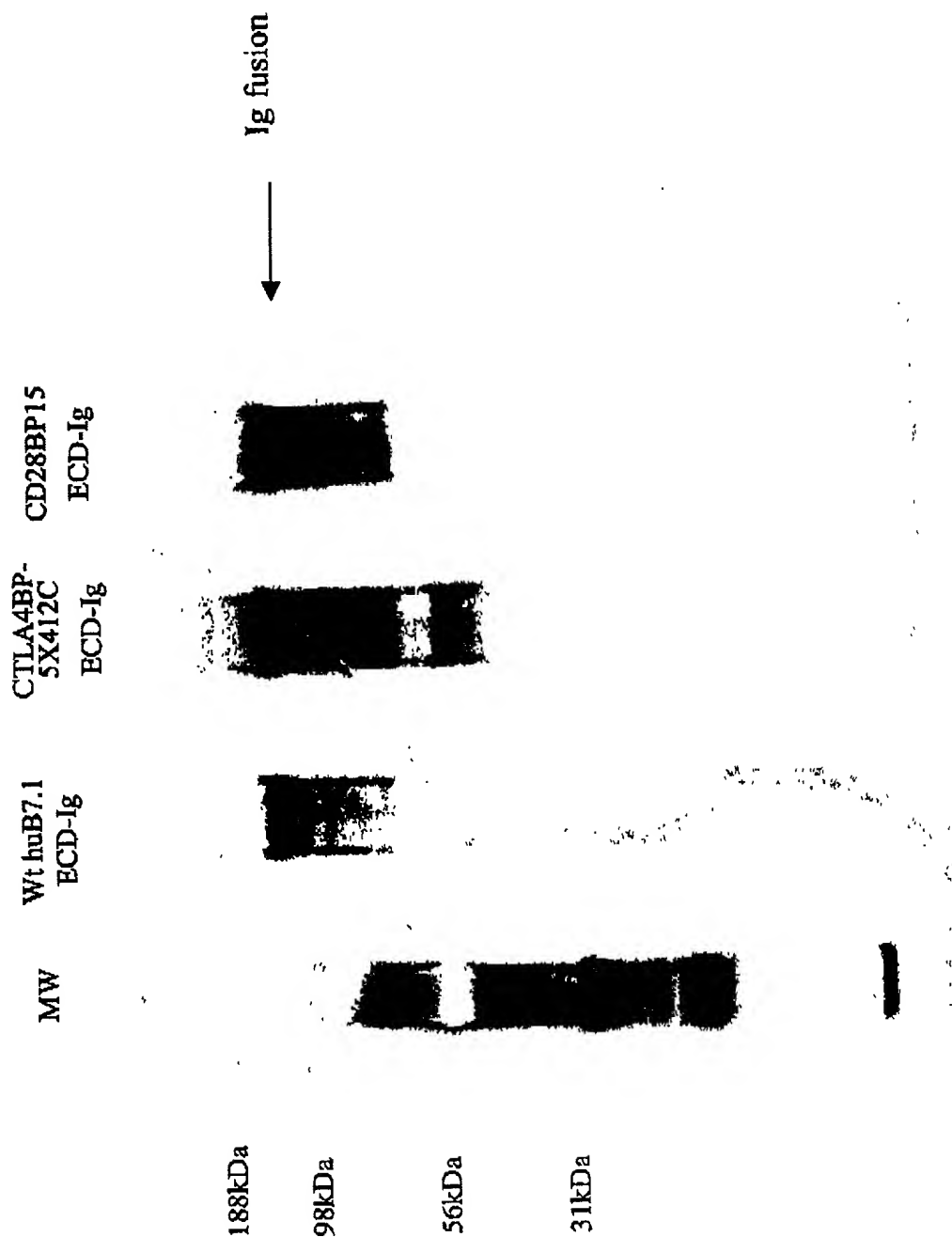


16
Figure 1

B7-1-ECD-Ig Fusion Expression Construct



Scale-up Production of wild-type soluble Human B7.1-, CTLA4BP 5X4-12C-, and CD28BP-15 ECD-Ig Fusion Proteins



Expression of CTLA-4-BP-Ig and CD28-BP-Ig Proteins

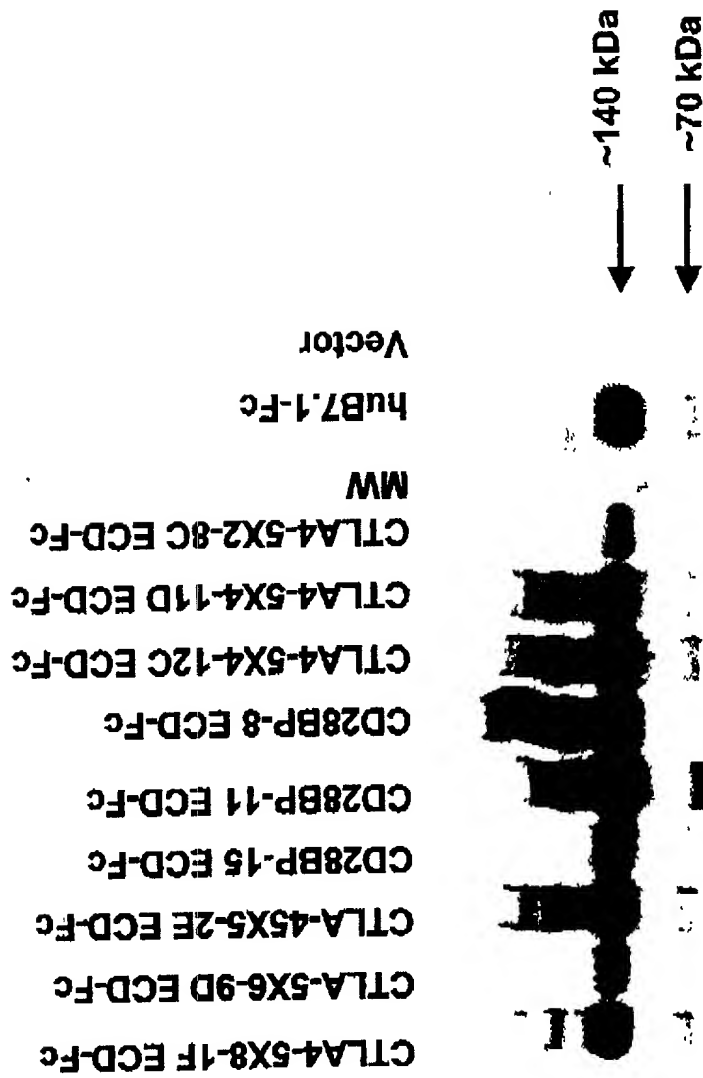
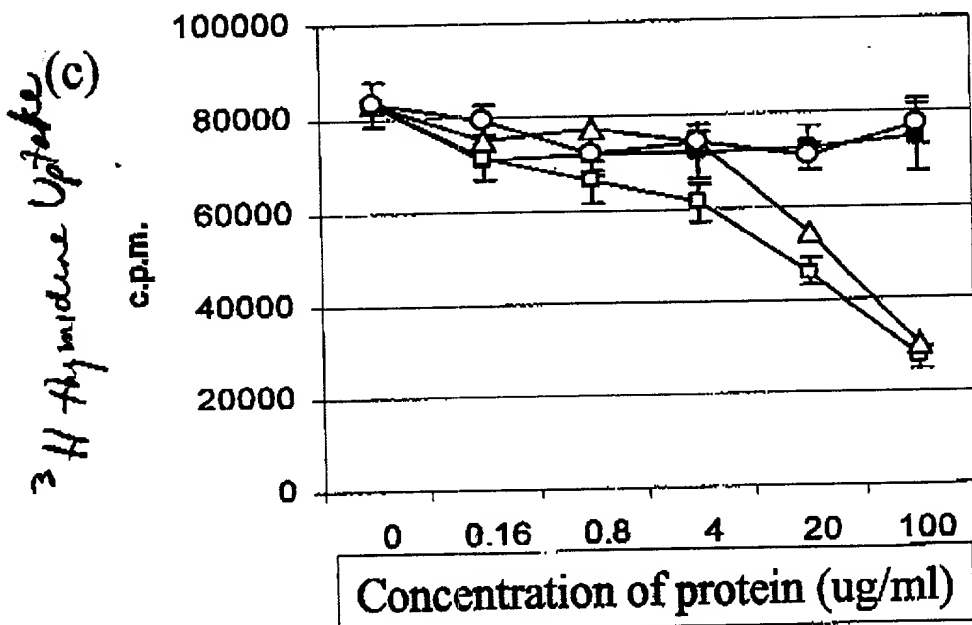
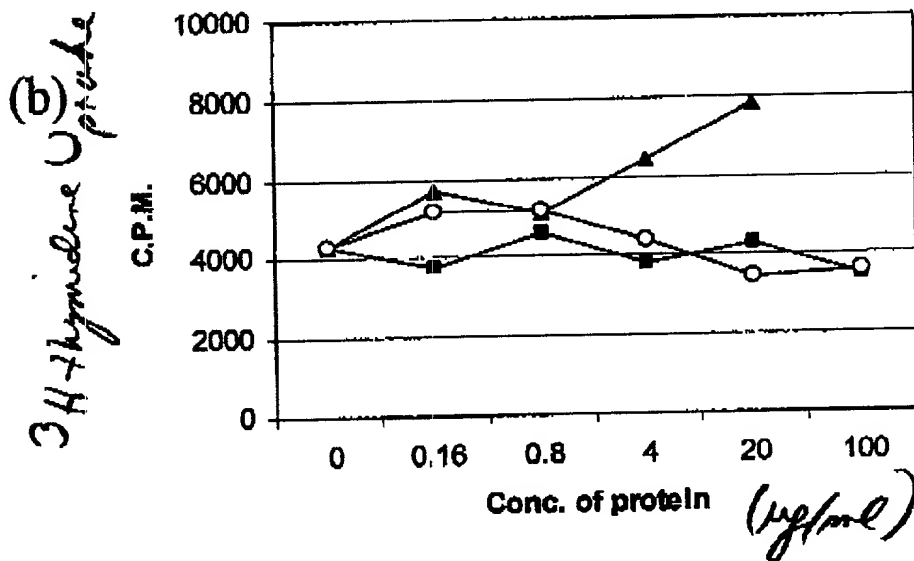
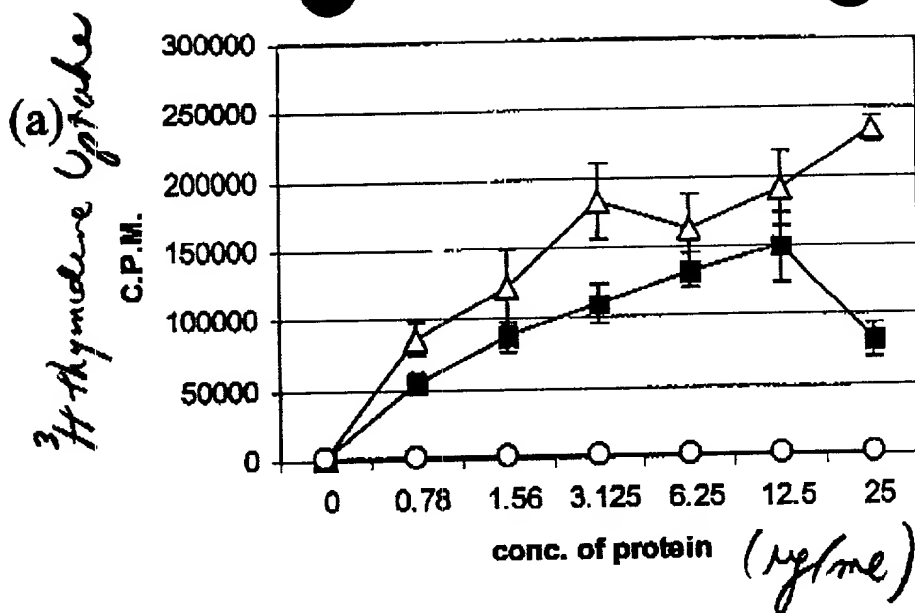


Figure 1

102250"4288860

Figures 20A-
20C



3 H^+ , molene Uptake

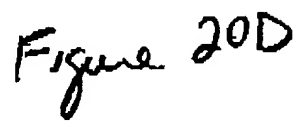
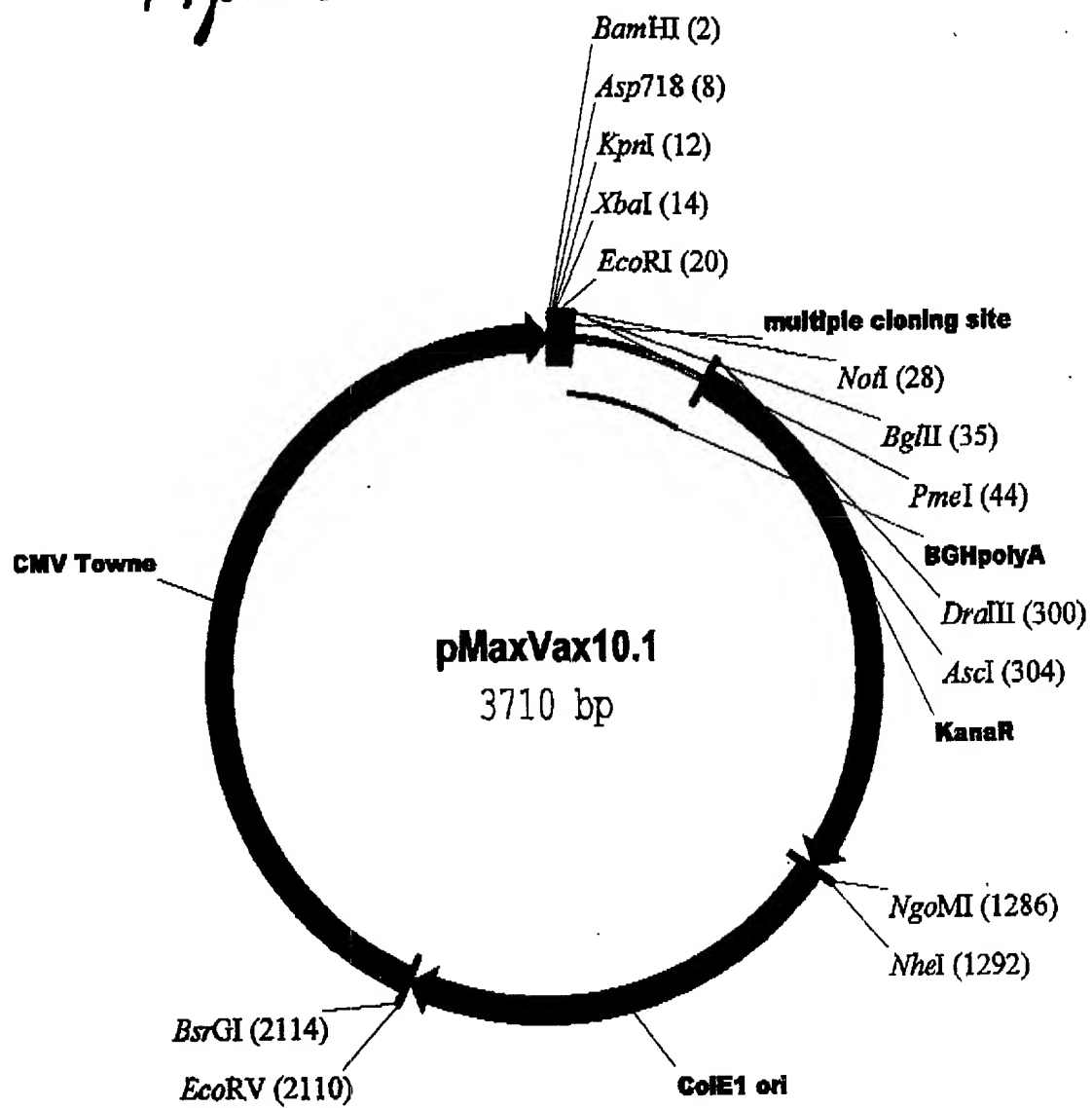


TABLE 1	
Summary of the results of the 1998-1999 survey of the 100 most common diseases and conditions in the United States	
Disease/condition	Prevalence (%)
All diseases/conditions	
1. Arthritis	10.1
2. Hypertension	9.8
3. Diabetes	7.5
4. Heart disease	6.8
5. Chronic obstructive pulmonary disease	6.5
6. Depression	6.2
7. Asthma	5.9
8. Back pain	5.6
9. Cancer	5.3
10. Chronic kidney disease	5.0
11. Alzheimer's disease	4.7
12. Chronic liver disease	4.4
13. Chronic pain	4.1
14. Chronic fatigue syndrome	3.8
15. Chronic sinusitis	3.5
16. Chronic bronchitis	3.2
17. Chronic heart failure	2.9
18. Chronic pancreatitis	2.6
19. Chronic thyroid disease	2.3
20. Chronic renal failure	2.0
21. Chronic hepatitis	1.7
22. Chronic colitis	1.4
23. Chronic ulcer	1.1
24. Chronic sinusitis	0.8
25. Chronic osteoporosis	0.5
26. Chronic glaucoma	0.2
27. Chronic epilepsy	0.1
28. Chronic schizophrenia	0.1
29. Chronic bipolar disorder	0.1
30. Chronic personality disorder	0.1
31. Chronic anxiety disorder	0.1
32. Chronic depression	0.1
33. Chronic insomnia	0.1
34. Chronic headache	0.1
35. Chronic dizziness	0.1
36. Chronic tinnitus	0.1
37. Chronic vertigo	0.1
38. Chronic balance disorder	0.1
39. Chronic ear pain	0.1
40. Chronic nosebleed	0.1
41. Chronic sore throat	0.1
42. Chronic cough	0.1
43. Chronic asthma	0.1
44. Chronic bronchitis	0.1
45. Chronic emphysema	0.1
46. Chronic pneumonia	0.1
47. Chronic tuberculosis	0.1
48. Chronic HIV/AIDS	0.1
49. Chronic hepatitis B	0.1
50. Chronic hepatitis C	0.1
51. Chronic liver failure	0.1
52. Chronic kidney failure	0.1
53. Chronic heart failure	0.1
54. Chronic stroke	0.1
55. Chronic Alzheimer's disease	0.1
56. Chronic Parkinson's disease	0.1
57. Chronic Huntington's disease	0.1
58. Chronic Tay-Sachs disease	0.1
59. Chronic Gaucher's disease	0.1
60. Chronic Fabry's disease	0.1
61. Chronic Hunter's disease	0.1
62. Chronic Hurler's disease	0.1
63. Chronic Marfan's syndrome	0.1
64. Chronic Ehlers-Danlos syndrome	0.1
65. Chronic osteoarthritis	0.1
66. Chronic rheumatoid arthritis	0.1
67. Chronic psoriasis	0.1
68. Chronic eczema	0.1
69. Chronic acne	0.1
70. Chronic alopecia	0.1
71. Chronic vitiligo	0.1
72. Chronic alopecia areata	0.1
73. Chronic alopecia totalis	0.1
74. Chronic alopecia universalis	0.1
75. Chronic alopecia excelsa	0.1
76. Chronic alopecia areata	0.1
77. Chronic alopecia totalis	0.1
78. Chronic alopecia universalis	0.1
79. Chronic alopecia excelsa	0.1
80. Chronic alopecia areata	0.1
81. Chronic alopecia totalis	0.1
82. Chronic alopecia universalis	0.1
83. Chronic alopecia excelsa	0.1
84. Chronic alopecia areata	0.1
85. Chronic alopecia totalis	0.1
86. Chronic alopecia universalis	0.1
87. Chronic alopecia excelsa	0.1
88. Chronic alopecia areata	0.1
89. Chronic alopecia totalis	0.1
90. Chronic alopecia universalis	0.1
91. Chronic alopecia excelsa	0.1
92. Chronic alopecia areata	0.1
93. Chronic alopecia totalis	0.1
94. Chronic alopecia universalis	0.1
95. Chronic alopecia excelsa	0.1
96. Chronic alopecia areata	0.1
97. Chronic alopecia totalis	0.1
98. Chronic alopecia universalis	0.1
99. Chronic alopecia excelsa	0.1
100. Chronic alopecia areata	0.1

Figure 21



Figures 22A-22B

